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The mouse macrophage-specific glycoprotein defined by monoclonal antibody F4/80: characterization, biosynthesis and demonstration of a rat analogue

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SUMMARY

F4/80, a mouse macrophage-specific membrane marker defined by a rat monoclonal antibody, was precipitated by a rabbit antiserum raised against partially purified mouse antigen. The antiserum, when tested against a variety of mouse tissues and cells, bound only to, and was cytotoxic for, macrophages, and it precipitated a similar macrophage-specific protein from rat cells. The F4/80 antigen is a glycoprotein of apparent molecular weight (MW) 150,000, and was labelled biosynthetically with [^{14}C]glucosamine. Neuraminidase treatment removed small amounts of sialic acid, and tunicamycin and 2-deoxyglucose both inhibited antigen synthesis. Pulse/chase labelling with [^{35}S]methionine demonstrated a precursor of 110,000 MW. Proteinase treatment of intact cells cleaved the molecule to an initial 100,000, and then to an 80,000 MW fragment. Without reduction, the MW of the molecule was unchanged by proteinases. These studies indicate that the F4/80 antigen consists of at least two domains linked by disulphide bridges, of MW 80,000 and 20,000. Both domains are extracellular.

INTRODUCTION

The rat monoclonal antibody (MAb) F4/80 recognizes a plasma membrane antigen that is uniquely restricted to mouse macrophages and macrophage-like cell lines (Austyn & Gordon, 1981; Hirsch, Austyn & Gordon, 1981; Nussenzweig *et al.*, 1981). This specificity of the F4/80 makes it a valuable reagent in mouse systems, and it has been used in immunocytochemical studies to identify macrophages *in situ* (Hume & Gordon, 1985), and in indirect radioimmunoassays of tissue extracts to estimate the macrophage content of the tissue (Lee, Starkey & Gordon, 1985). The MAb does not cross-react with macrophages of other species, however, and has no cytotoxic activity.

The antigen recognized by the F4/80 MAb is a membrane protein of apparent MW 150,000, which can be radiolabelled by surface iodination, or biosynthetically with [^{35}S]methionine (Austyn & Gordon, 1981). In view of its importance as a specific macrophage marker, it was of interest to characterize the F4/80 antigen further, and attempt to raise an antiserum recognizing an analogous antigen in other species.

Abbreviations: MAb, monoclonal antibody; PBS, phosphate-buffered saline; RPM, resident peritoneal macrophages; TPM, thioglycollate-elicited peritoneal macrophages.

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MATERIALS AND METHODS

Antibodies and reagents

Chemicals were from Sigma Chemical Co., Poole, Dorset, except as follows: *Vibrio cholerae* neuraminidase (Calbiochem, Cambridge Bioscience, Cambridge); *Arachis hypogea* (peanut) lectin (P.L. Biochemicals Inc., Milwaukee, WI); wheat germ agglutinin (Vector Laboratories Inc., Burlingame, CA); culture media (Flow Laboratories Ltd, Rickmansworth, Herts); radiochemicals (Amersham International, Aylesbury, Bucks). Antibodies used as concentrated culture supernatants or purified by affinity chromatography were F4/80, a rat IgG2b MAb (Austyn & Gordon, 1981); Mac-1, clone M1/70 from Dr T. Springer (Harvard Medical School, Boston, MA); and the chromatographically purified F(ab′)$_2$ fragments of affinity-purified rabbit anti-rat IgG, MRC OX12, a mouse anti-rat kappa chain MAb from Dr S. V. Hunt (Sir William Dunn School of Pathology, Oxford) and horse anti-rabbit IgG from Dr A. F. Williams (MRC Cellular Immunology Unit, Oxford). Antibodies were covalently linked to Sepharose CL-4B (Pharmacia Fine Chemicals AB, Uppsala, Sweden), using cyanogen bromide (Axen & Ernback, 1971). Rabbit complement was Lo-tox M from Sera-Lab Ltd, Crawley Down, Sussex.

Cells

All cells were incubated at 37° in 5% CO_2, heat-inactivated fetal calf serum (FCS), and 20 μg/ml gentamycin. Resident (RPM) or thioglycollate-elicited (TPM) peritoneal macrophages were from Swiss Pathology Oxford mice aged 8–10 weeks, untreated AO rats, or guinea-pigs (Austyn & Gordon, 1981), and purified

by overnight adherence in Dulbecco's modified Eagle's medium (DMEM)/10% FCS. Human peripheral blood monocytes were isolated, and cultured for 6 days as adherent monolayers (Mokoena & Gordon, 1985). The cell line J774.2 (Ralph, Prichard & Cohn, 1975), free of mycoplasma, was grown in suspension as Spinner cultures at $0{\cdot}5$–$1{\cdot}0 \times 10^6$ cells/ml in Iscove's medium/8% FCS. Cells, >98% viable by trypan blue exclusion, were harvested by centrifugation at 350 *g* for 10 min.

Radiolabelling of cells

Adherent TPM were labelled in 60-mm petri-dishes after 30–48 hr in culture, and J774·2 in suspension. Cells were washed with Eagle's minimum essential medium (MEM) then labelled biosynthetically overnight in methionine-free MEM containing 60 μCi/ml L-[^{35}S]methionine, or in glucose-free DMEM containing 10 μCi/ml D[U-^{14}]glucosamine HCl. All media were supplemented with 8% dialysed FCS, 2% FCS. For pulse/chase experiments, cells were cultured in methionine-free MEM for 1 hr, then with 120 μCi/ml of L-[^{35}S]methionine for 30 or 60 min before being washed three times with PBS and incubated for 30 or 60 min with methionine-free MEM, supplemented as before but with the addition of 100 μM unlabelled methionine.

Enzyme treatment

Radiolabelled cells were washed three times with PBS containing 10 mM azide, incubated at 37° for 30 min with 2 ml of PBS/azide containing trypsin, (1, 5, 10, 25 or 50 μg/ml), chymotrypsin, (50 or 100 μg/ml) or elastase, (1, 5, 10, 25, 50 or 100 μg/ml), and enzyme activity inhibited with 25 μl Pms-F (75 mM in isopropanol) for a further 5 min at room temperature. For neuraminidase treatment, PBS adjusted to pH 6·5 was used, and incubation for 30 min at 37° was with 0, 0·05, 0·10 or 0·20 U/ml of *Vibrio cholerae* neuraminidase.

Immunoprecipitation and gel electrophoresis

Cells were washed repeatedly with ice-cold PBS, lysed with PBS containing 1% Triton X-100, 10 mM EDTA, 3 mM iodoacetic acid, 3 mM Pms-F (lysis buffer), scraped from the culture dish with a rubber policeman and kept on ice for 30 min before being centrifuged at 9000 *g* for 8 min to remove cell debris. Lysates (50 μl from 10^6 cells) were precleared by incubation for 1–2 hr with 10 μl of preformed rabbit anti-rat IgG IgG/protein A–Sepharose complex, or rat IgG–Sepharose, or protein A–Sepharose (50% v/v in PBS/azide), then centrifuged at 9000 *g* for 30 seconds. Fifty microlitres of precleared supernatant were incubated overnight with 5 μl FCS and 1–5 μg of MAb followed by 10 μl of rabbit anti-rat IgG IgG/protein A–Sepharose for 1 hr, or 10 μl of F4/80 MAb–Sepharose (50% v/v in PBS/azide) or 10 μl KSl antiserum (diluted five times with PBS) followed by incubation for 1–2 hr with 10 μl of protein A–Sepharose. All incubations were at 4°. In each case the adsorbed immune complexes were recovered by centrifugation, washed (Mellman *et al.*, 1980) and dissolved by boiling for 5 min in electrophoresis sample buffer. SDS–gel electrophoresis was in the Ammediol–chloride–glycine buffer system (Barrett, Brown & Sayers, 1979) in 0·7 mm thick gradient acrylamide gels (5–20% T, 2·6% C). [^{14}C]methylated protein mixture was run as MW standards. Gels were fixed, dried or treated for fluorography and dried, and autoradiography performed at −70° using pre-fogged Kodak No-Screen Film, NS-2T. Densitometry was by use of a Joyce–Loebl Mk III CS microdensitometer (double-beam recording).

Immunoblotting was essentially as described by Towbin, Staehelin & Gordon (1979). Staining of rat tissues with the rabbit polyclonal antibody was by the biotin–avidin system (Hume & Gordon, 1985), and fluorescence-activated cell sorter analysis as described by Austyn & Gordon (1981).

Assays for F4/80 antigen

This was either by indirect competition assay (Lee *et al.*, 1985) or a direct assay on nitrocellulose. In the latter, antigen-containing samples (4 μl) were applied at 1-cm intervals to a sheet of nitrocellulose, which was allowed to dry, then incubated at room temperature with gentle shaking as follows: (i) 2 hr in PBS containing 3% bovine serum albumin, 0·2% Tween 20 and 10 mM azide (PBS/BSA/Tween 20), (ii) 1 hr with first antibody diluted in the same, (iii) fresh PBS/BSA/Tween 20 (four changes, total time 45 min), (iv) 1 hr with [^{125}I]labelled OX12 diluted in PBS/BSA/Tween 20 to 2–5×10^5 c.p.m./ml, and (v) washed as in (iii) above. The nitrocellulose was dried and 1-cm squares were counted in a gamma counter.

Partial purification of F4/80 antigen

J774.2 cells (4×10^9) were washed and lysed with two volumes of freshly made lysis buffer, centrifuged and the supernatant filtered. Antigen was purified from the lysate by affinity chromatography on a column of F4/80 MAb–Sepharose essentially as described by Brown & Williams (1982). Antigen concentration was monitored, and purity of fractions assessed by microradio-iodination (Hughes, Colombatti & August, 1983). The eluted antigen was contaminated with various proteins of lower MW which could not be removed by a second round of affinity chromatography.

Immunization of rabbit KS1

Partially purified F4/80 antigen (five units in the competition assay) in 0·5 ml of equal volumes of PBS/0·1% DOC and Freund's complete adjuvant was injected intramuscularly at Time 0 and at 3 weeks. The rabbit was bled at 4 weeks. A booster injection of antigen as above except using incomplete Freund's adjuvant was given at 5 months, and two bleeds (each approximately 50 ml) were taken 8 and 11 days later. These bleeds were pooled and used as the rabbit antiserum KSl.

RESULTS

Preparation and characterization of a polyclonal antiserum to the F4/80 antigen

The rabbit antiserum KSl, raised in response to partially purified mouse F4/80 antigen (see Materials and Methods), was shown to precipitate only the same antigen as the rat monoclonal (Fig. 1, Tracks B—D). In binding studies to fixed target cells, in fluorescence-activated cell sorting with live peritoneal and bone marrow cell populations, and in immunocytochemical studies on a variety of mouse tissues, the polyclonal antiserum stained only macrophages. The cytotoxic activity of the rabbit antiserum, tested in several experiments using rabbit complement, killed more than 90% of mouse TPM, compared with controls using either antibody or complement alone.

A single cell assay, using antibodies linked by $CrCl_2$ to sheep erythrocytes, was used to investigate whether the rabbit antiserum recognizes the same or different epitopes to the F4/80

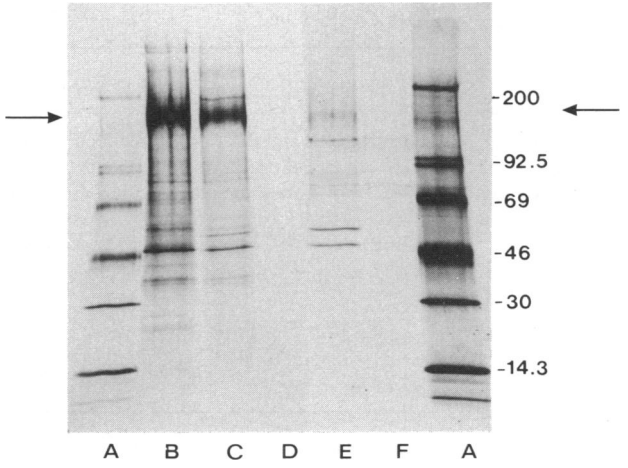

Figure 1. Immunoprecipitation by rabbit antiserum KSl of F4/80 antigen from mouse macrophages, and the cross-reacting antigen from rat macrophages. Mouse TPM or rat RPM were labelled with [^{35}S]methionine, lysed and immunoprecipitated with either F4/80 MAb or KSl rabbit antiserum. Immunoprecipitates were analysed by SDS–gel electrophoresis followed by fluorography. Samples were [^{14}C]protein standards (A), mouse TPM lysate immunoprecipitated with F4/80 MAb–Sepharose (B), or KSl rabbit antiserum and protein A–Sepharose (C), or rabbit preimmune serum and protein A–Sepharose (D), and rat RPM lysate, immunoprecipitated with KSl and protein A–Sepharose (E) or rabbit preimmune serum and protein A–Sepharose (F). Arrows indicate the F4/80 antigen, and the MW values ($\times 10^{-3}$) are on the right.

MAb. Binding to adherent TPM of the rat monoclonal or rabbit polyclonal antibodies was detected using erythrocytes coated with either rabbit anti-rat IgG F(ab′)$_2$ or horse anti-rabbit IgG F(ab′)$_2$. Controls showed no cross-reaction between the two detection systems. Preincubation of the target cells with the rabbit KSl antiserum blocked subsequent binding of the rat MAb by 90–100%, while in a control experiment the rabbit preimmune serum gave no inhibition. In contrast, preincubation of the target cells with the F4/80 MAb had little effect on the subsequent binding of the KSl antiserum.

When tested against cells from other species, the rabbit antiserum did not bind to cultured human monocytes, nor to guinea-pig TPM. It did bind, however, to rat RPM and precipitated a molecule which gave a diffuse band in SDS–gel electrophoresis similar to that given by the mouse F4/80 antigen, but of slightly lower MW (Fig. 1, Tracks E and F). In immunocytochemical staining of sections of rat tissues, the antiserum appeared to bind only to macrophages.

Heterogeneity of the F4/80 antigen

Antigen detected by immunoblotting of detergent lysates of TPM or J774.2 cells gave a diffuse band in SDS–gel electrophoresis as shown previously for radiolabelled immunoprecipitates (Austyn & Gordon, 1981). The F4/80 band remained diffuse, even when electrophoresis was in the presence of 2·5 M urea to ensure complete unfolding of the protein. Antigen was not detectable by immunoblotting with either rat monoclonal or rabbit polyclonal antibodies, if electrophoresis was in reducing conditions. From its electrophoretic mobility the antigen has an apparent MW of about 150,000 although this varies between cell types, the antigen from TPM having a slightly slower mobility than that from the cell line. In addition, the mobility of antigen from a given cell type was found to vary between experiments, corresponding to MW values of 122,000–159,000 for TPM for example. Similar variability has been reported for other membrane glycoproteins, including the macrophage glycoprotein Mac 3 (Ho & Springer, 1983).

Glycoprotein nature of the F4/80 antigen

The behaviour of F4/80 antigen in SDS–gel electrophoresis suggested that it might be a glycoprotein. In order to test this directly, TPM were labelled biosynthetically with [^{14}C]glucosamine, and immunoprecipitation demonstrated that the F4/80 antigen was labelled with [^{14}C]glucosamine (Fig. 2), as were both the α and β chains of the Mac-1 antigen, confirming previous results for Mac-1 (Kurzinger & Springer, 1982). The nature of the link between carbohydrate and protein portions of the F4/80 molecule was investigated by use of tunicamycin, which blocks the incorporation of N-linked carbohydrate into glycoproteins (Kuo & Lampen, 1974), and 2-deoxyglucose, which competitively blocks incorporation of both N- and O-linked carbohydrate (Schmidt, Schwarz & Scholtissek, 1974; Lehle & Schwarz, 1976).

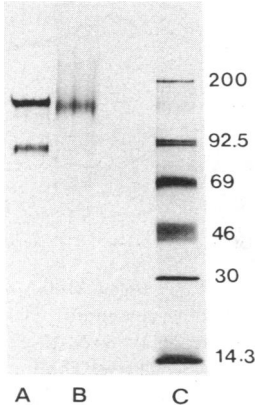

Figure 2. Biosynthetic labelling with [^{14}C]glucosamine of F4/80 and Mac-1 antigens. Mouse TPM were labelled with [^{14}C]glucosamine, lysed, and the immunoprecipitates analysed by SDS–gel electrophoresis followed by fluorography. Samples were material immunoprecipitated with Mac-1 (A), or F4/80 MAb (B) and [^{14}C]protein standards (C).

Tunicamycin at 5 μg/ml and 2-deoxyglucose at 0·35 mg/ml both markedly reduced the amount of immunoprecipitable F4/80 antigen, with lower doses having lesser effects. General protein synthesis as assessed by the incorporation of [^{35}S]methionine was unaffected by either inhibitor at these concentrations.

Further investigation of the nature of the carbohydrate in F4/80 was attempted by treating the cells with neuraminidase to remove terminal sialic acid residues, or by measuring binding of radiolabelled lectins to immunoprecipitated antigen. All levels of neuraminidase caused the same slight increase in mobility of the F4/80 antigen in gel electrophoresis, indicating the removal of small quantities of sialic acid. None of the lectins tested—concanavalin A, lentil lectin, wheat germ agglutinin, peanut

lectin, BS4 or *Lotus tetragonolobus*—bound to the F4/80 antigen, whether or not the cells had been pretreated with neuraminidase.

Biosynthesis of F4/80 antigen

Pulse/chase experiments with J774.2 cells in suspension, or adherent TPM, demonstrated the presence of a precursor molecule precipitated by both monoclonal and polyclonal antibodies. The densitometer traces reproduced in Fig. 3 clearly show the transfer with time of radiolabel from the 110,000 MW precursor to the mature 150,000 MW antigen. For both J774.2 cells and TPM, total precipitable radioactivity and the percentage present as precursor and mature forms of F4/80 were calculated from densitometry of autoradiographs of the gels. The results (see Table 1) show a $t_{1/2}$ for conversion of the precursor to the mature form of about 30 min in TPM, and slightly less in the J774.2 cell line. In order to assess whether cells contain an intracellular pool of F4/80 antigen or whether antigen, once synthesized, is rapidly transported to the plasma membrane, antigen levels in J774.2 cells were assayed on intact cells suspended in PBS, or on an equal number of cells lysed in detergent-containing lysis buffer. Cell lysates were found to contain antigen levels only 25% higher than those obtained with intact cells, providing no evidence of a large intracellular pool of antigen.

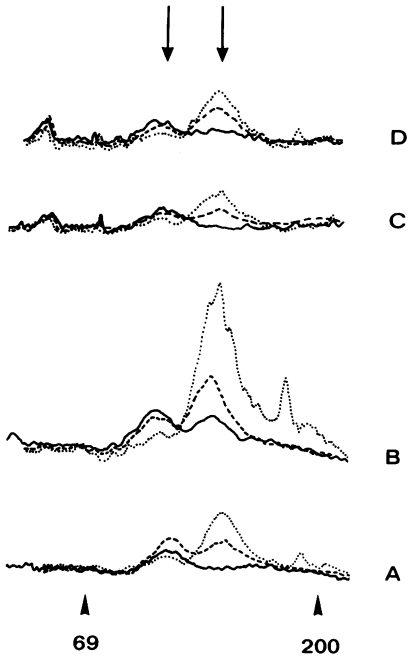

Figure 3. Biosynthesis of F4/80 antigen. Mouse TPM were radiolabelled with a pulse of [^{35}S]methionine, chased with cold methionine, lysed and immunoprecipitated with either F4/80 MAb–Sepharose or KSl rabbit antiserum and protein A–Sepharose. The immunoprecipitates were analysed by SDS–gel electrophoresis, fluorography and densitometry. Samples were pulsed for 30 min (A and C) or 60 min (B and D), then chased with unlabelled methionine for 0 (——), 30 (– – –) or 60 (. . . .) min. Antigen was immunoprecipitated with either F4/80 (A and B) or KSl antiserum (C and D). MW values ($\times 10^{-3}$) are indicated at the bottom, and the two arrows mark the position of the mature F4/80 antigen and its 110,000 MW precursor.

Table 1. Biosynthesis of F4/80 antigen in J774.2 cells and TPM

		J774.2			TPM		
Pulse (hr)	Chase (hr)	Total (units)	Precursor (% of total)	Mature (% of total)	Total (units)	Precursor (% of total)	Mature (% of total)
0·5	0	8·9	65	35	9·4	72	28
0·5	0·5	16·8	37	63	19·8	50	50
0·5	1·0	28·2	23	77	22·5	17	83
1·0	0	17·2	55	45	24·3	54	46
1·0	0·5	24·2	38	62	37·0	34	66
1·0	1·0	15·2	25	75	60·1	17	83

Cells were labelled *in vitro* with a pulse of [^{35}S]methionine, then chased with cold methionine and lysed. F4/80 antigen immunoprecipitated from the lysate was analysed by SDS–gel electrophoresis, and the amount of radioactivity in the bands representing the mature and precursor forms of the antigen assessed by densitometry. Total radioactivity of both forms together was expressed in arbitrary units, and the radioactivity of each form expressed as a percentage of the total.

Structure of the F4/80 antigen and organization of the molecule in the plasma membrane

Trypsin treatment of TPM gave the results expected from previous work (Fig. 4a). At levels of trypsin greater than or equal to 5 μg/ml, a single cleavage product of apparent MW 80,000 was obtained, and 50 μg/ml were needed for 100% cleavage. The recovery of labelled antigen, assessed by densitometry of the gels, was 55, 38, 42, 35 and 20% at 1, 5, 10, 25 and 50 μg/ml of trypsin, respectively, suggesting that at all levels of trypsin, a considerable proportion of antigen was completely degraded.

In contrast to the results with trypsin, chymotrypsin and pancreatic elastase gave an intermediate cleavage product of apparent MW 100,000 (Fig. 4b, chymotrypsin not shown). Thus, 5 μg/ml of pancreatic elastase gave about 50% conversion of the intact antigen molecule to the MW 100,000 first cleavage product, 25 μg/ml of enzyme gave 50% conversion of this fragment to the MW 80,000 second cleavage product, and 100 μg/ml of enzyme was needed for total conversion to the 80,000 fragment. If, however, the proteolytically cleaved antigen was run under non-reducing conditions (Fig. 4c), the MW of the antigen molecule was unchanged even at the highest enzyme level when, under reducing conditions, the molecule could be shown to be completely converted to the 80,000 MW fragment. Elastase treatment of cells labelled biosynthetically with [^{14}C]glucosamine gave essentially the same cleavage products, indicating that both the 100,000 and 80,000 MW fragments contain carbohydrate, and that no other large fragments containing carbohydrate or labelled with [^{35}S]methionine are released. Antigen immunoprecipitated from cells labelled with [^{35}S]methionine then incubated with trypsin (2·5 μg/ml) or pancreatic elastase (5 μg/ml) gave no detectable fragments in SDS–gel electrophoresis under reducing conditions.

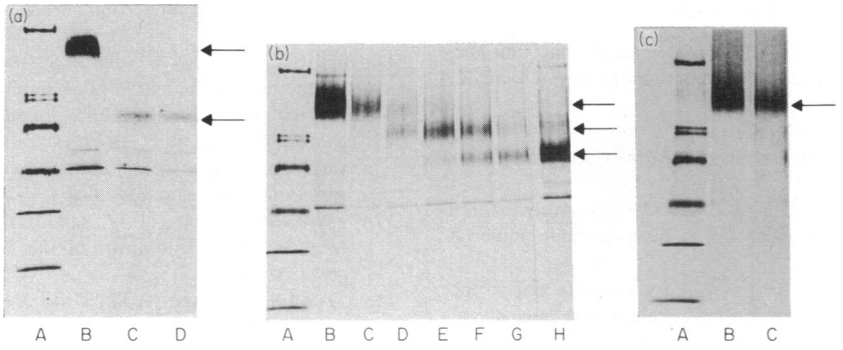

Figure 4. The effect of proteinases on F4/80 antigen. Mouse TPM were radiolabelled with [^{35}S]methionine, treated with proteinases for 30 min at 37°, lysed and immunoprecipitated with F4/80 MAb. Immunoprecipitates were analysed by SDS–gel electrophoresis and fluorography. (a) The effect of trypsin: samples, electrophoresed in reducing conditions, were [^{14}C]protein standards (A), and immunoprecipitates from untreated cells (B), or cells treated respectively with 10 or 50 μg/ml of trypsin (C, D). Arrows mark the intact antigen and the 80,000 MW fragment. (b) The effect of elastase: samples, electrophoresed in reducing conditions, were [^{14}C]protein standards (A), and immunoprecipitates from untreated cells (B) or cells treated with, respectively, 1, 5, 10, 25, 50 or 100 μg/ml of pancreatic elastase (C–H). Arrows mark the intact antigen and the 100,000 and 80,000 MW fragments. (c) The effect of elastase without reduction: samples were electrophoresed in non-reducing conditions, and were [^{14}C]protein standards (A), and immunoprecipitates from untreated cells (B) or from cells treated with 100 μg/ml of pancreatic elastase (C). Arrows mark the F4/80 antigen.

DISCUSSION

The rabbit antiserum KSl, although it was raised against the intact F4/80 molecule, and recognizes several other epitopes in addition to the one detected by the MAb, binds only to F4/80 and a related macrophage-specific rat protein. This suggests that the rest of the F4/80 molecule is macrophage-specific as well as the epitope recognized by the rat MAb. In view of its cytotoxic activity, the rabbit antiserum provides a valuable reagent for specific killing of mouse and rat macrophages.

The results described give several clues to the structure of the F4/80 antigen. It is a membrane glycoprotein containing N-linked oligosaccharide units with small amounts of terminal sialic acid residues. The structure of the oligosaccharide units has not been identified, although the absence of binding to any of the lectins tested rules out some of the common structures known from other glycoproteins.

The difference between the biosynthetic intermediate and the mature antigen is likely to be due to processing of the oligosaccharide side-chains, and the large change in MW, an increase of 40,000 representing nearly 30% of the mature molecule, would seem to suggest that the F4/80 antigen contains a very high percentage of carbohydrate. Results with the LDL receptor, however, suggest that alterations in the processing of oligosaccharide side-chains can cause disproportionately large changes in MW (Cummings *et al.*, 1983).

The fact that cleavage of the antigen by trypsin, chymotrypsin or pancreatic elastase produced similar or identical fragments, despite the different substrate specificities of the three enzymes, suggests that the molecule is organized into at least two domains of apparent MW 20,000 and 80,000 linked by regions of polypeptide chain particularly sensitive to proteolytic attack by virtue of their tertiary structure, and bridged by disulphide bonds. Since these sites must be accessible to proteinases, both domains must be extracellular, and not buried in the membrane.

Cleavage of the antigen to the 100,000 and 80,000 MW fragments leaves a substantial portion of the molecule unaccounted for. We were unable to label F4/80 biosynthetically with [^{14}C]palmitate, nor to remove the antigen from the cell surface by treatment with the phospholipase which breaks the lipid/protein bond in Thy-1 (Low & Kincade, 1985), so it is unlikely that the missing fragment is covalently bound lipid. It seems probable that partial degradation of the missing portion of the molecule is the explanation, although, since even after elastase treatment the whole F4/80 molecule remained covalently linked by disulphide bridges, extensive degradation is unlikely.

Although the rabbit antiserum to F4/80 failed to demonstrate any cross-reactivity with other cellular antigens either on macrophage or on other cell types, apart from the rat homologue, other membrane proteins have been described that share some properties with the F4/80 antigen. A major membrane glycoprotein of guinea-pig macrophages, gp160, shares with F4/80 its MW, its reduced levels of synthesis in activated macrophages (Remold-O'Donnell & Lewandrowski, 1982), and sensitivity to trypsin. Unlike F4/80, however, gp160 is bound to lentil lectin (Remold-O'Donnell, 1980), although this could represent species variation. The CSF-1 receptor like F4/80 is restricted to macrophages, and gives a diffuse band of similar MW in SDS–gel electrophoresis (Guilbert & Stanley, 1980; Sherr *et al.*, 1985). However, recent studies using the F4/80 MAb and purified CSF-1 have shown that the F4/80 antigen and CSF-1 receptor are distinct (P. Crocker and R. Stanley, unpublished).

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